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Published in:
Book of Abstracts. DTU's Sustain Conference 2015

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

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Parkinson's disease is characterized by insufficient dopamine in the brain, a neurotransmitter involved in the motor function. One of the future ideas for treatment is cell replacement therapy. We have shown that pyrolysed 3D carbon micropillars induce spontaneous differentiation of human neural stem cells (hNSCs) into dopaminergic neurons and that they can also be employed for detecting dopamine release from maturing neurons attached to them [1]. Here, we report 3D carbon nanopillars, fabricated through colloidal lithography, with even more pronounced effect on the differentiation process.

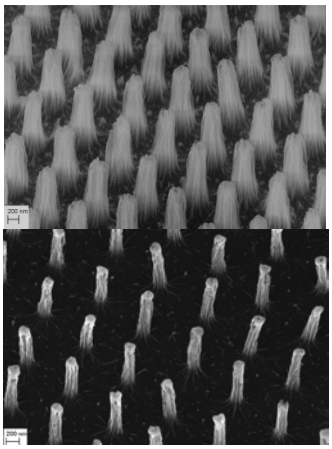

Figure 1: SEM image of nanopillars before (top) and after (bottom) pyrolysis

The 3D carbon nanopillars were obtained using 1 µm beads and etching time of 20 min, leading to structures with a height of 1.2 µm and a diameter of 450 nm (before pyrolysis) and a height of 600 nm and a width of 200 nm after pyrolysis (figure 1).

Carbon nanopillars were employed as substrate for cell-growth and differentiation after plasma treatment (surface wettability) and poly-L-lysine coating (cell adhesion). Even without adding differentiation factors, differentiation into dopaminergic neurons is observed. SEM imaging, immunocytochemistry of TH and amperometry confirm that most cells are TH-positive and dopaminergic. Dopamine exocytosis was induced using KCl and monitored using amperometry on electrodes with nanopillars 48 h after cell seeding, in the absence of differentiation factors and 10 days after cell seeding, in the presence of differentiation factors. For comparison, flat carbon surfaces were tested in parallel. Amperometry measurements were conducted on electrodes with nanopillars 48 h after cell seeding, in the absence of differentiation factors and 10 days after cell seeding, in the presence of differentiation factors. For comparison, flat carbon surfaces were fabricated and tested in parallel.

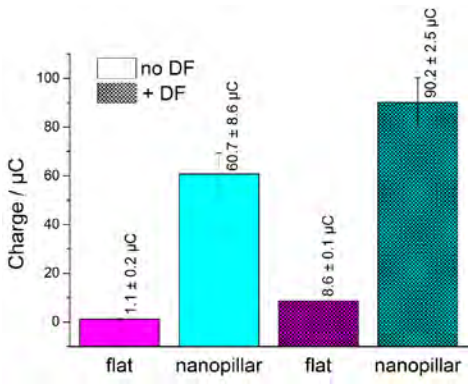

Figure 2: Charge comparison for dopamine exocytosis for flat and nanopillar carbon surfaces with or without of differentiation factors

Compared to flat carbon surfaces, nanopillar electrodes show a high increase in currents, although the surface area increase is negligible (figure 2).

We here show carbon surfaces with nanopillars that induce a remarkable spontaneous differentiation of human neural stem cells into dopaminergic neurons and that they can be employed as electrodes for dopamine detection from mature dopaminergic cells and that they show a much better electrochemical response compared to flat carbon surfaces and 3D carbon micro-pillars previously reported [1].

References: 1. L. Amato et. al., Pyrolysed 3D-Carbon Scaffolds Induce Spontaneous Differentiation of Human Neural Stem Cells and Facilitate Real Time Dopamine Detection, *Advanced Functional Materials*, **2014**, Vol. 24, Issue 44, 7042-7052.